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## THE MICROVIVISECTION METHOD.

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The dissection of living cells has hitherto proved inadequate for a study of the physical properties of protoplasm and of its structural components. This is owing largely to a lack of means for the manipulation of dissecting instruments under a sufficiently high magnification of the microscope.

Before and since Barber first devised his mechanical pipette-holder for the isolation of bacteria, various ingenious methods for the dissection of microscopic objects have been described. They all, however, fall short of Barber's method, because, with them, only comparatively low powers can be used, since the dissecting needles must operate *between* the objective and the tissue to be dissected. On the other hand, by using needles instead of micro-pipettes, Barber's instrument has been converted into an excellent microdissection apparatus. The needles operate in a shallow hanging drop containing the cells to be dissected which are pressed against the *undersurface of a coverslip* in a moist chamber. There being no obstacle above the coverslip, oil immersion lenses may be used for observation.

In an article appearing as long ago as 1859, Dr. H. D. Schmidt, of Philadelphia, describes in detail an excellent "microscopic dissector" consisting of a base to be fastened on the stage of a microscope with a number of clamps to hold instruments, each clamp possessing three movements controlled by screws. A lever fastened in one of the clamps holds the tissue in place. Fine scissors, knives or steel needles are fastened in the other clamps. By turning the various screws, the instruments can be brought into place and be operated with remarkable accuracy. Dr. Schmidt worked with the tissue, the instruments and the lower lens of the objective immersed in water or diluted alcohol. The full results of his investigations with this instrument were not published until 1869, owing to interruptions due to the Civil

War, during which his drawings and manuscript were burned in a conflagration in Washington. In his later paper he has two illustrations showing a liver cell being torn in two by needles.

In 1887 Chabry described an apparatus in which a glass needle was held in a sheath in such a way as to allow the needle to be pushed to any prescribed distance into the bore of a glass capillary tube. The bore of the capillary was made just large enough to admit a single ovum and hold it in place. By proper adjustments, the needle could be pushed to any depth in the ovum. The apparatus was placed on the stage of a microscope and Chabry was able to injure locally a *Strongylocentrotus* ovum while under observation. Chabry's instrument has been used for experimental embryological work and is described in detail in Ehrlich's "Enzyklopädie für mikroskopischen Technik," 1910.

In 1907 J. F. McClendon first described his "mechanical finger," which consisted of an ordinary Spencer mechanical stage with an additional screw to allow of movements in three different directions and a clamp to hold a needle or pipette. With this apparatus, McClendon was able to suck the nucleus out of a *Chaetopterus* egg. In 1909 he described an improved form with which he dissected certain Protozoa.

In 1912 an apparatus was described by Tschachotin. It consists of a clamp attached to the side of the objective of a microscope. In the clamp is fastened a needle which curves so as to project under the objective and is so adjusted that the tip lies in the focus of the objective. The needle point is lowered into the object to be dissected simply by bringing the object into view under the microscope and lateral tears are made on moving the object with the slide by means of the mechanical stage.

In 1904 Barber first described his method of using a hanging-drop in a moist chamber for isolating microorganisms. A fuller account appeared in 1907 in which he first describes a mechanism for holding micropipettes and where he mentions Montrose T. Burrows as having assisted him much in its design. Elaborations of his instrument appeared in his papers of 1908 and 1911. In 1911 he suggested the possibility of the use of his instrument for cell dissection and for investigation on fertilization and heredity problems. His paper of 1914 is a detailed and precise descrip-

tion of his technique and is of the utmost value for the prospective worker in micro-dissection. As he developed his method principally for the use of pipettes and also because his latest paper appeared in a publication not readily obtainable, I have endeavored to give here a description of the method with modifications and new developments in the application of the method to microvivisection.<sup>1</sup>

A simple form of Barber's apparatus is shown in Fig. 1. The moist chamber, which is open at one end and with sides from 8 to

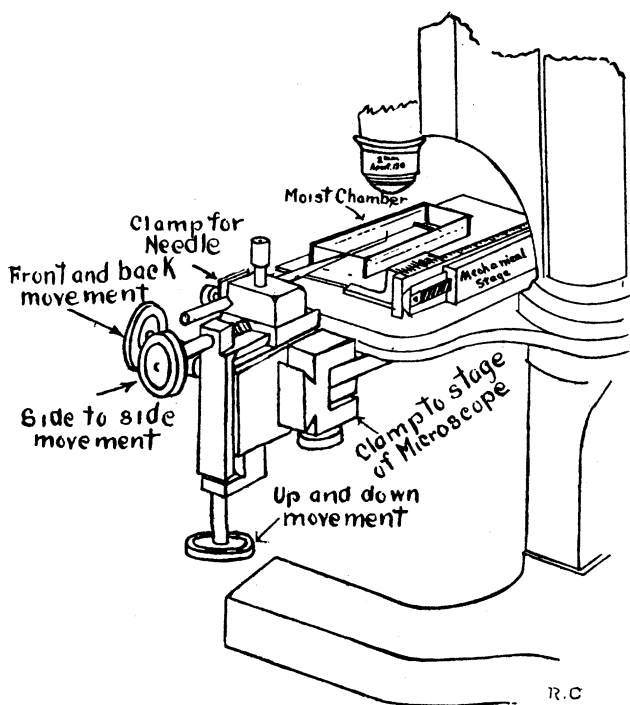


FIG. 1. Barber's single three-movement pipette holder, glass needle and moist chamber arranged to illustrate method of dissecting cells in a hanging drop under the highest magnification of the microscope. (Substage of microscope omitted in drawing.)

<sup>1</sup> In 1912, G. L. Kite first applied Barber's apparatus to microdissection, which quickly resulted in the publication of several papers. Kite was to have prepared an article on the method when his health unfortunately broke down. Publications on microdissection have appeared as follows: 1912, Kite (on the fertilization membrane) in *Science*, N. S., XXXVI.; Kite and Chambers (on chromosomes and nucleus) in *Science*, N. S., XXXVI. 1913, Kite (on permeability of cells) in *Biol.*

12 mm. high, is placed on the microscope so that it may be moved about with the mechanical stage. The chamber is roofed over with a carefully cleaned coverslip, on the under surface of which, the specimen is mounted in a shallow hanging drop of a physiologically indifferent fluid. The dissecting needle is made by drawing out one end of a piece of glass tubing and bending it at right angles two or three millimeters from the pointed tip. The needle-holder, a mechanism allowing of three movements, is clamped to one side of the microscope stage and the needle is adjusted so that it may project into the moist chamber with its tip pointing up into the hanging drop. By proper adjustment the cell to be dissected and the point of the needle are brought into the same focal field. The three movements of the needle, permitted by the needle-holder, and the two movements of the moist chamber, by means of the mechanical stage, give the experimenter ample opportunity to carry on dissection under the highest magnification of the microscope.

#### THE INSTRUMENT.

The holder accommodated to carry only one pipette or needle is illustrated in Fig. 1. It is useful in many ways and is comparatively inexpensive. Its disadvantage lies in the fact that the resistance of the cell-substance to the needle may overcome the inertia of the cell, so that, instead of tearing through the cell, the needle simply drags it about. However, by careful manipulation and by the possible use of a viscous dissection-medium much can be done. Many marine ova and germ cells in general are very soft in consistency and, if the drop in which they are immersed be shallow enough, the surface tension of the drop flattens them against the coverslip and is sufficient to hold the cell. The single holder can also be successfully used for injecting material into or extracting material out of a cell. In addition to the movement of the needle, manipulation is greatly aided by the

BULL., XXV., and (on protoplasm) in *Amer. Jour. Physiol.*, XXXII., 1914, Chambers (on the nucleus) in *Science*, XL.; Kite (structural transformations of blood cells) in *Jour. Inf. Dis.*, XV. 1915, Chambers (on the germ cell) in *Science*, XLI.; and (on protoplasm) in *Lancet-Clinic*, March 27, Cincinnati; Kite (on cell permeability) in *Amer. Jour. Physiol.*, XXXVII. 1917, Chambers (on protoplasm) in *Amer. Jour. Physiol.*, XLIII.; and (on the cell aster) in *Jour. Exp. Zool.*, XXIII.

use of the mechanical stage by means of which the moist chamber may be moved in two directions.

The double holder, formerly manufactured by the Fowler shops of the University of Texas, is twice as expensive as the single holder. It possesses, however, the advantage that two needles or pipettes may be used simultaneously. Each needle can be moved independently of the other and either needle may be replaced by a pipette. The manufacture of the holder has recently been duplicated by the mechanician of Wesleyan University under the direction of Dr. H. B. Goodrich with modifications of his and my suggestion. In setting up the apparatus, Dr. Goodrich introduced the useful innovation of having the shelf to which the instrument is clamped on a stand independent of the microscope. The two stands are then clamped together on a common base. The operator can thus shift the position of the instrument placing it at will in front of or on either side of the microscope.

Fig. 2 illustrates the double holder as I have it set up. The attachment of the instrument to the front of the microscope stage facilitates manipulation of the screws with both hands. In the illustration, a Spencer Lens Company lamp replaces the substage mirror. As the heat of the lamp, however, produces undue evaporation and subsequent condensation in the moist chamber, it is preferable to use the mirror. This necessitates raising the microscope-stand on a block of wood so that the mirror can be lowered below the microscope base so as to receive light unobstructed by the lower screws of the dissecting instrument.

The needle-carrier with its groove (Fig. 2, *a*), in which the arm of the needle lies, is twice as long as in Barber's original instrument. This extra length assists one in giving a straight even movement to the needle when it is being pushed into the moist chamber. After the needle has been thus adjusted by hand approximate to its proper position, the plate *b* is pushed over the arm of the needle and the screw *c* tightened to clamp the needle. Further adjustment of the needle is carried out by the screws *e*, *f*, *g* and *h* under the low and high powers of the microscope.

In preparing the shelf on which the instrument is to be clamped (Fig. 2, *j*) care must be taken that it be low enough to bring the top of the carrier flush with the upper surface of the microscope

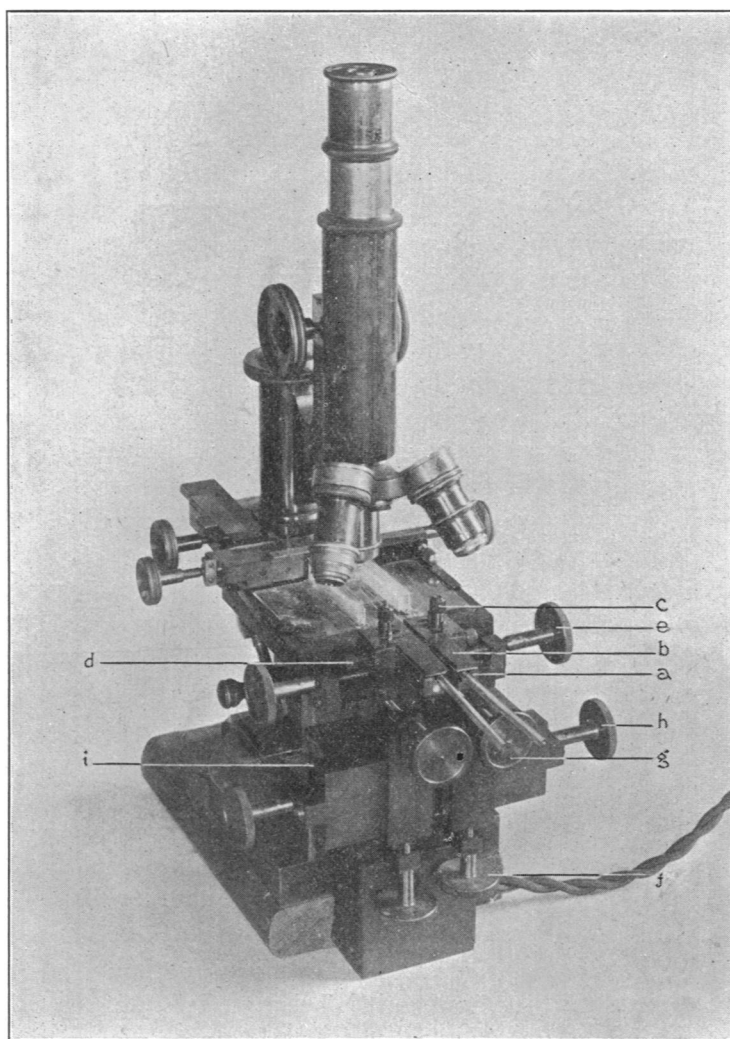


FIG. 2. Double pipette holder set up for microdissection. *a*, Carrier with groove to hold needle. *b*, brass plate which slips sideways over the groove in which needle lies. (The undersurface of the plate is covered with blotting paper to give it resiliency where it comes into contact with the glass needle.) *c*, set screw to clamp plate on carrier. *d*, screw which, on loosening, allows carrier to be set at any angle. *e-h*, screws to move the needle (*e*—side to side, *f*—up and down, *g*—in and out, *h*—side to side for both carriers together). *j*, shelf to which instrument is clamped.

stage. This is in order to enable one to use a moist chamber of the minimum height (see below).

An important factor in the manufacture of the microdissection instrument is that the two horizontal movements run true and keep the needle point always in the same plane. Otherwise, at a critical moment in the dissection, a reversal of a horizontal screw may either suddenly lower the needle-point out of focus or jam it against the coverslip and break it. Unfortunately, the last model put out by the University of Kansas is deficient in this respect. The existence of lost motion in the screws can be taken care of in the manipulation but if the needle tip does not run true in a horizontal plane the peace of mind of the operator may be sorely tried!

#### THE MOIST CHAMBER.

The moist chamber (Fig. 3) is a glass slide to which are cemented strips of glass in such a way as to form a box open at the top and at one end. For cement Canada balsam may be used.

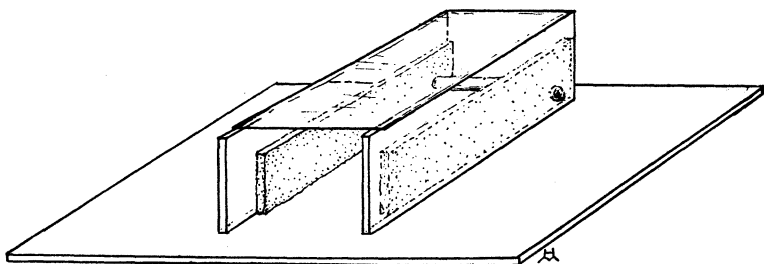


FIG. 3. Moist chamber for microdissection.

A shallow trough, to hold water, is made with a narrow glass strip set across the floor of the chamber 8 or 9 mm. from the closed end. This and other small strips set outside the chamber serve to reinforce the walls. Care must be taken that the upper edges of the chamber be even and level when placed on the stage of the microscope. A carefully grease-free cleaned coverslip with a hanging drop containing the objects to be dissected forms the roof of the chamber. The roof is sealed on with vaseline smeared along the upper edges of the walls of the chamber.

The height of the chamber is conditioned by the focal distance of the condensor used and by the minimum working room that



one must have in which to manipulate the needles. For routine work it may be well to have a chamber of fairly large dimensions disregarding the condensor as it is surprising how much illumination a condensor will give beyond its focal distance. However, for critical work, it is imperative to have the height of the chamber such that the objects on the undersurface of the coverslip be close to the focus of the condensor. My condensor has a working focal distance of almost 9 mm. and the chambers I use are 45 mm. long, 22 mm. wide and 9 mm. high.

The moist chamber is lined on the sides with blotting paper leading from the trough to carry the water along the length of the chamber so as to furnish a large moist surface. The liquid in the trough and the blotting paper should be isosmotic with the liquid used for the hanging drop so that evaporation be equalized and the hanging drop not become diluted.

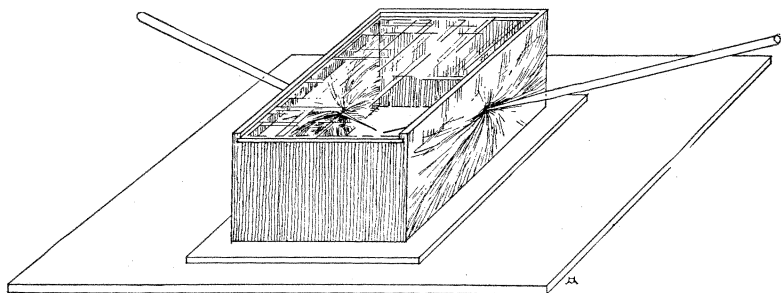


FIG. 4. Moist chamber for preliminary teasing of tissue.

For the dissection of the cellular elements of somatic tissues, it is necessary to do some preliminary teasing under an ordinary dissecting microscope. This should be done in a moist chamber of some kind. The tissue is teased on a coverslip which is then inverted and placed on the microdissection moist chamber.

A handy moist chamber (Fig. 4) can be made out of a shallow box with dimensions approximately  $7 \times 5 \times 3$  cm., with a top and bottom of glass and the two longer sides of thin rubber sheeting. The coverslip with the tissue to be teased is placed within the box and the teasing is carried out with needles projecting through the rubber sheeting. For the tissue of warm-blooded animals both the preliminary teasing and the microdissection should of course, be carried on in warm boxes.

Tissue cultures are grown on square coverslips. The slips are then placed on the moist chamber for microdissection, the parts of the chamber not roofed over being previously covered with slips of glass.

#### THE NEEDLES.

These are made from glass tubing with an outside diameter of about 4–6 mm. and a lumen of 3–5 mm. The technic is readily mastered with soft glass tubing but needles made of hard glass are more durable.

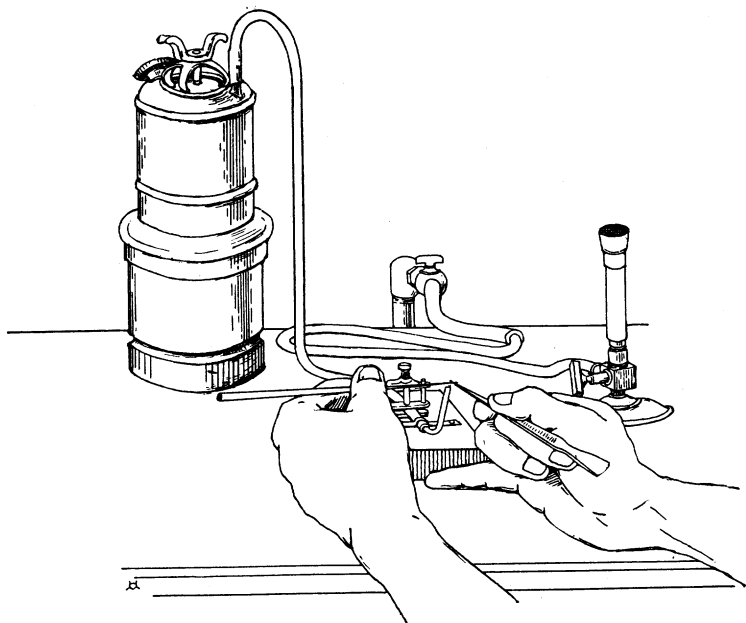


FIG. 5. Method of making the capillary pipettes and microdissection needles.

The needle points are made over a minute flame. A serviceable micro-burner with an acetylene generator is shown in Fig. 5. The microburner is made out of a piece of hard glass tubing bent almost at right angles and with one end closed except for the smallest possible aperture that will retain a flame. This is done by heating the end and pinching it with a pair of forceps. When set up the tip of the burner should be at a height of about 5–6 cm. above the surface of the table. The size of the flame is regulated by the screw pinch-cock set on the rubber tube connecting the burner with the gas generator.

As a source for the flame acetylene is to be preferred to ordinary illuminating gas because with the former a narrower flame can be obtained without undue clogging. The acetylene generator in the figure is part of an old bicycle lamp. A more convenient way is to have the acetylene in a small compression tank which can be recharged at a very small cost. If, however, ordinary gas is to be used, one may much improve the gas by passing it through alcohol or benzene.

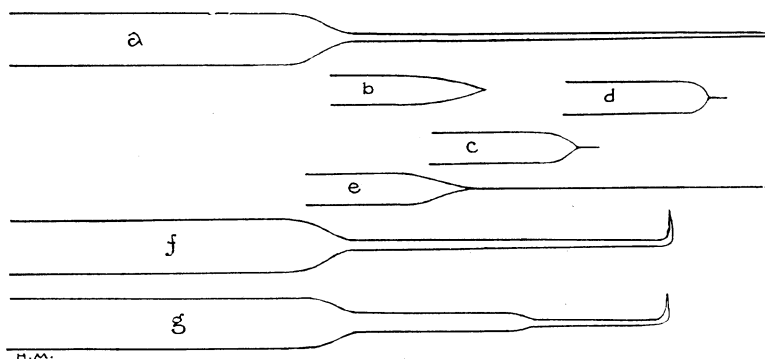


FIG. 6. Stages in making needles. *a*, glass tube with one end drawn out into a capillary. *b*, good tapering point. *c* and *d*, serviceable points. *e*, tip drawn out into a hair. *f*, completed microdissection needle. *g*, needle made on a relatively stout shank.

The method of procedure in making the glass needle is as follows:

1. In an ordinary Bunsen burner (or a Mecca burner if hard glass be used) draw out one end of a piece of glass tubing into a straight capillary about 0.3–0.5 mm. in outside diameter (Fig. 6, *a*). Prepare a supply of such pieces with the capillary ends at least 6 cm. long.

2. Lower the flame of the microburner to the smallest flame that will remain lighted. The microburner should be protected from draughts of air and in semi-darkness so that the flame may show up to best advantage.

3. Hold the shank of the glass tube in the left hand and, with a pair of fine forceps held in the right, grasp the capillary at a point about 5 cm. from the shank. Bring the portion of the capillary next the forceps over the flame and at right angles to it at a point just over the flame (Fig. 5). Pull gently with the

forceps and, when the glass begins to soften, lift it slowly from the flame and pull with the forceps slightly more than at first, but not too strongly. The hands should remain on the table during the process and the pulling and lifting done by turning them slightly outward. The capillary will separate with a slight tug—a feeling much like that experienced when a taut thread, held in the fingers, is parted in a small flame. If the point is properly made, it will appear as shown in Fig. 6, *b*, *c* and *d*. This capillary has sufficient rigidity and it comes to a very fine point. The tip is closed, but the lumen extends almost to the end. It is evident that everything depends upon the amount of heat used and the timing of the pull, and that these must vary slightly with the height of the flame and the diameter of the capillary. With a little experience, one can usually tell when a proper point is made by the peculiar feeling described above. If too little heat is used and the pull made too suddenly, the capillary may part with a snap and the tip be found broken off short often forming a serviceable pipette. If too much heat is used, the capillary is apt to be drawn into a long hair (Fig. 6, *e*).

4. After a suitable point is made, the end of the capillary is turned up at right angles (Fig. 6, *f*). This is done by holding the point of the capillary just back of the point above the small flame and pushing up the end with the tip of the forceps or with a needle. Not more than 5 mm. should be turned if it is to be used in a chamber not over 8 mm. in height. If a good point is made, *e. g.*, Fig. 6, *b*, it is not necessary to turn the end at exactly right angles. A pipette is made by jamming the tip of a needle against the cover glass in the moist chamber to break off the point.

The forceps used in the making of the needles should be of a size that can be firmly grasped in the hand. The fine tips should be accurately set and almost parallel one another for a few millimeters. In order to give the tips a good, grasping, resilient surface I dip them, before using, in a small vial of Sandarac varnish which may be obtained from a dentists' supply dealer. As the varnish burns if brought too close to the flame, it is well to use a second forceps or a needle for bending the glass capillary.

Fine points are not readily made from capillaries of a diameter

greater than .05 mm. Therefore, if the dissection requires a comparatively stiff shank for the dissecting needle, start with a glass tube possessing a thick capillary and draw out the tip into a thinner capillary. This thinner capillary may then be used for making the needle point (Fig. 6, *g*).

The facility in making the needle-points over the microburner increases, of course, with practice. In my case, I make at times twenty or thirty within an hour, at other times none, or only one or two at the most. A supply may be kept on hand on a stand made of two horizontal layers of wire netting, the meshes of which admit the base of the needles. It is well to mark off the stand into compartments to receive the various types of needles that are made.

Another good method for making the needle points is that of Chabry ('87). The tip of a capillary pipette is brought into contact with a heated mass of glass, (or any incandescent body to which glass will adhere) and suddenly drawn away. For an incandescent body Chabry used the blade of a platinum knife of a surgical instrument for thermocautery. The glass capillary was held by the operator in a groove on a stand a few centimeters from the platinum blade, which was fastened so as to be immovable. An assistant controlled the heating of the platinum blade. As soon as it heated to a dull glow, the operator slid the capillary on its groove until the tip touched the glowing metal when it was instantly slid back. The sliding of the capillary in a stationary groove insures a tapering to a point in a straight line with the long axis of the capillary. In his paper Chabry discusses this method in some detail, pointing out ways of avoiding difficulties in the technique ('87, pp. 175-178). Chabry also suggested the use of insect mouth parts, annelidan bristles and the spicules of sponges for the tips of microdissection needles. Barber suggested fine-pointed needle crystals, or the sharp, stiff hairs taken from the body of a house fly. A pipette with a fairly large opening is made and the hair or crystal is drawn partially into it. The fine point projecting from the tip of the pipette is then used as a probe or dissecting needle. Such needles could be used only on very delicate soft objects.

Needle points may also be made by grinding one end of a fine

wire. To produce chemical effects in a cell, McClendon ('09) used a copper wire ground to a point and further sharpened by erosion in acid. The chemical injury produced by metal needles limit their use for the dissection of living cells.

For experiments in cutting cells, one must take into account the fact that many cells will lose their more or less fluid contents if a gap be torn in their surface. If, however, the side of a fine needle can be brought to press the cell against the coverslip, a constriction will result which may cut the cell into two intact pieces. For cutting soft-bodied ova and protozoa this can be done with a form of needle depicted in Fig. 7. The needle tip

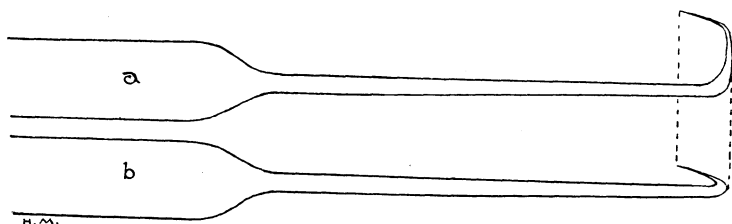


FIG. 7. Needle with tip curved back to almost an horizontal plane. *a*, lateral view. *b*, view from above.

is bent back rather than forward to lessen the chances of breaking the tip when adjusting the needle in the moist chamber and also when the needle needs to be cleaned (see below). The tip should not point directly back but slant off to one side (see Fig. 7, *b*), so that the shank of the capillary will not be in the way of the light coming up from below. The angle at which the needle is bent must be ascertained by successive trials, for, if it be bent too near to a horizontal plane, the tapering tip will bend away from the cell instead of cutting into it. If the angle be too far from the horizontal plane, the cell will slip along the needle and escape. When the proper angle is obtained, one can cut *Arbacia* and *Asterias* ova into pieces of almost any required size with considerable ease. A clean cut through an unfertilized sea-urchin egg or a fertilized egg, before the fertilization membrane has become too tough, can be best made by bringing the egg between the horizontal arm of the needle and the lower surface of the hanging drop (Fig. 8). On lowering the needle out of the drop,

the arm of the needle passes through the egg cutting it cleanly in two.

A good needle, when once made and proven to be resilient and strong, may be used repeatedly. Mucinous material which soon

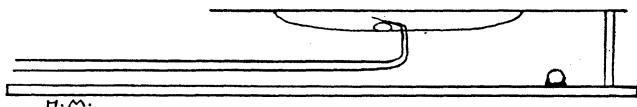


FIG. 8. Side view of moist chamber to show method of cutting an egg cell in two with needle shown in Fig. 7.

covers its tip so as to render it useless can be got rid of by immersing the needle successively in a strong acid and a strong alkali.

#### CELL INJECTION.

Barber, in his two papers ('11 and '14) gives a full description of his admirable mercury method for injection. With it one can inject microscopically minute doses of a solution into any part of a cell. The advantage of the pressure produced by the mercury over that by air lies in the slow driving force of expanding mercury which can be almost instantly arrested when the dose injected is considered sufficient. On the other hand, if air pressure be used, the air in the injection tube will compress enormously as the resistance of the bore at the tip of the pipette is too great to overcome. If the injection does occur it may go with a rush usually followed by a volume of air which destroys the cell being experimented upon. Notwithstanding this, it is surprising what delicate work may be done simply with air pressure. An example of this is the extraction of the nucleus from a mature *Arbacia* egg and its subsequent injection into another egg. For the injection of substances, however, where the amount must be strictly controlled, the mercury method is essential.

The extraction of material out of a cell is comparatively easy to carry out, as the capillary attraction in the lumen of a micro-pipette (the inside diameter of which may be anywhere from 2 micra up) is quite sufficient for the purpose. For injection purposes the difficulty lies in securing the force to overcome this capillary attraction.

## SOURCE OF LIGHT AND LENSES.

A most serviceable light for microscopic work is the 100-watt horseshoe filament, Edison, Mazda, nitrogen lamp with the blue daylight glass. This lamp, when set in a frosted milk-white glass globe, gives a white light very restful to the eye.

In selecting lenses care should be taken to procure those possessing the maximum focal length together with the greatest numerical aperture. For this reason the Zeiss apochromatic 4 mm. dry lens with a N. A. 0.95 and the 3 mm. oil immersion lens with N. A. 1.40 are excellent for microdissection or for the study of living tissue in general. It is to be remembered that the equivalent focus of a lens does not correspond with the working focal distance.

A good substage condensor with a working focal distance of 8–9 mm. can be made by removing the top lens of the Leitz triple-lens, centering condensor. The beam of light issuing from it completely fills the back lens of a 4 mm. objective with N. A. 0.65 and more than half of a  $1/12$  oil immersion objective with a N. A. 1.30. This gives enough definition for such detailed cytological work as the study of the chromomeres in grasshopper germ-cells and of the types of protoplasmic granules in marine ova. One may obtain very good condensors with a long working focal distance from those used in projection apparatus where a cooling trough is inserted between the condensor and the microscope stage.

## SETTING UP THE INSTRUMENT.

The instrument is first clamped to the microscope and the moist chamber, with the edges of its walls smeared with vaseline, placed in the mechanical stage. The needle is then adjusted as follows: Place the arm of the needle in the groove of the carrier with the dissecting tip extending into the moist chamber. Push the plate of the carrier (*b*, in Fig. 2, page 126) over the arm of the needle and tighten the set screw *c* just enough to allow one to push the needle with a straight and even movement. The needle is now adjusted by hand so as to bring the tip within the field of a low power objective. The set screw is then tightened to clamp the needle in place. Further centering of the needle is made by



the screws *e*, *f*, *g* and *h*, under the low and high powers of the microscope. Finding the tip of the needle under high powers is facilitated by using an ocular containing cross hairs.

The coverslip with its drop containing the tissue to be dissected is now inverted over the moist chamber. The cells to be dissected are brought into the field of the microscope. The tip of the needle is then raised by the screw *c* until brought into view first under low and then under high power. The apparatus is now ready for dissection.

In conclusion I wish to suggest to the prospective worker in this field that he use the utmost conservatism in his interpretations of observed effects produced in microdissection. One must constantly keep in mind the extraordinary instability of living cell structures.

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